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Alkaloid variations in *Catharanthus roseus* seedlings treated by different temperatures in short term and long term

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Abstract: A study was conducted to investigate the effects of high temperature on variations of alkaloid metabolism in *C. roseus* seedlings in Key Laboratory of Forest Plant Ecology, Northeast Forestry University, Heilongiang, China. 60-day-old *C. roseus* seedlings with 3-4 pairs of leaves were incubated in chambers with temperature of 30ºC and 40ºC for short-term heat shock experiment and 20ºC, 25ºC and 35ºC for long-term experiment. The contents of vindoline, catharanthine, vinblastine and vincristine in *C. roseus* leaves and root were checked at different temperatures in short term (1−6 h) and long term (1−16 d). Results showed that under short-term heat shock, the contents of vindoline, catharanthine and vinblastine in leaves of the seedlings were higher at 40ºC than at 30ºC, but after 6 h treatment, the contents of vindoline and catharanthine under the two temperatures came to the same level. Catharanthine was exclusively distributed in *C. roseus* roots and its content was increased by 40% after two hours incubation at 40ºC, while increased slowly at 30ºC incubation and reached the highest value at 6 h. In the Long-term experiment, concentrations of monomeric alkaloids catharanthine and vindoline were higher at 20ºC than at 25ºC and had a sharp increase under the condition of 35ºC. While for dimeric alkaloid, it showed that the higher the temperature, the earlier the peak value of vinblastine content appears. Vincristine had a continuous enhancement and attained 0.027 $mg \cdot g^{-1}$ at 16th day under 35ºC condition which was higher than those in the other conditions. It was concluded that high temperature could promote the accumulation of different alkaloids in *C. roseus* and the accumulation characteristic is highly related to treatment time.

Keywords: *Catharanthus roseus*; Temperature; Treatment time; Alkaloids

Introduction

Plants produce a rich diversity of secondary compounds, which seems not necessary for basic metabolism but appear to contribute to their environmental fitness and adaptability (St-Pierre *et al*. 1999). Alkaloids belong to a broad category of secondary metabolites and this class of molecule has traditionally been of interest due to the pronounced and various physiological activities in humans (Facchin 2001). Vinblastine, one kind of dimeric alkaloids from *Catharanthus roseus*, has been widely used in the treatment of cancer and received great attention for its metabolism and regulating mechanisms (De Luca *et al.* 1988). Vindoline and catharanthine are the other two crucial indole alkaloids existing in *C. roseus* and the enzymatically coupling of them can form vinblastine (Sottomayor *et al.* 1996).

Originally produced in Madagascar, the seedlings of *C. roseus* suffer from high temperature in short or long term. It has been reported that alkaloid accumulation was modulated by environmental temperature and high temperature preferred to induce alkaloid biosynthesis. Four hours incubation at 42ºC heat shock resulted in 3-hold accumulation of 10-hydroxycamptothecin in leaves of *Camptotheca acuminata* seedling (Zu *et al*. 2003). The detailed functions of increased alkaloids during heat shock remain to be uncovered although it was well known that alkaloids were involved with the stress processes of plants (Frischknecht *et al*. 1987; Vazquez-Flota *et al*. 2004). In this study, we checked the variation rules of vindoline, catharanthine and vinblastine in *C. roseus* seedlings under different temperatures in short term (hour) and long term (day) to determine their characteristics of alkaloid metabolism.

Materials and methods

Plant materials and treatment

C. roseus seedlings were grown in a growth chamber (CONVIRON, Canada) under the conditions that temperature was 28℃ in the day and 24℃ in the night, photoperiod was 16 in the day and 8 in the night, humidity was 60%. 60-day-old *C. roseus* seedlings with 3-4 pairs of leaves were treated with different temperatures. For short-term heat shock experiment, the involved seedlings were incubated in chambers with temperature of 30℃ and 40℃. For long-term experiment, the seedlings were transferred to different chambers with temperature of 20℃, 25℃ and 35℃. The contents of vindoline, catharanthine, vinblastine and vincristine in *C. roseus* leaves and root were checked at different temperatures in short and long term.

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Determination of alkaloids

The analysis of vindoline, catharanthine, vinblastine and vincristine were performed according to Aert *et al*. (1996). Fresh leaf powder (0.3 g) was dissolved in 10 mL absolute methanol. Alkaloids were extracted for 20 min using low-frequency ultrasonic technology (250 W, 40 kHz). Methanol extract was centrifuged at 15 000 r·min^{-1} for 10 min and concentrated to 1 mL for high performance liquid chromatography (HPLC, Jasco, VG, England) and this HPLC system was equipped with a Waters ODS C18 reversed-phase column (250 mm×4.6 mm) and a photodiode-array detector set up at 220 nm. Sample injection volume was 10 μL. Flow-rate was 1.5 mL·min^{-1}.

Results and analysis

Effect of high temperature on alkaloid metabolism in short-term treatment

The contents of vindoline, catharanthine and vinblastine in incubated seedlings were higher than that of controlled ones. Short term experiment under temperatures of 30ºC and 40ºC showed that the contents of vindoline, catharanthine and vinblastine in leaves of *C. roseus* seedlings were higher at 40ºC than at 30ºC, which indicated that high temperature favors the biosynthesis of alkaloids (Fig. 1). Contents of vindoline (Fig. 1a) and catharanthine (Fig. 1b) increased with treatment time (hour) under temperature of 30ºC and reached peak value at 6 h, 3.56 mg·g^{-1} for vindoline and 2.3 mg·g^{-1} for catharanthine. For 40ºC treatment, vindoline content peaked at 2 h and catharanthine content at 4 h. The ultimate contents of vindoline and catharanthine were basically same after 6-h treatment, although some differences were found in their change way under different temperatures. This result suggested that the variations of vindoline and catharanthine contents under short-term heat shock were involved with the rapid stress response process of plants. The concentrations of vinblastine at 30ºC and 40ºC did not arrived at the same levels after 6 h treatment, which indicated that the functions of vinblastine and its precursors might be distinguishing (Fig. 1c).

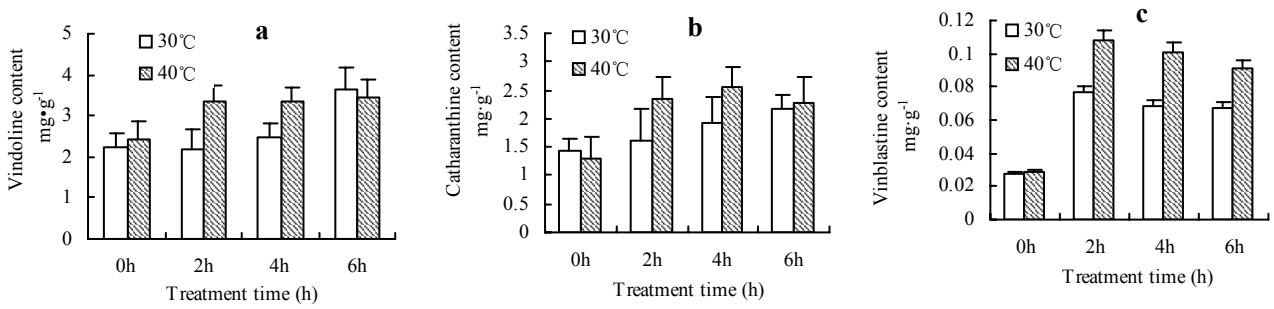

Fig.1 Alkaloid contents in leaves of *Catharanthus roseus* seedling incubated with different temperature

Error bars represent ±SD calculated from four independent experiments.

Catharanthine is only detected in roots and its content was as low as 0.08 mg·g^{-1}. Exposure to high temperature induced obvious promotion of catharanthine content in roots. Catharanthine content in roots of the seedling was increased by 40% after two hours incubation at 40ºC, while it increased slowly at 30ºC incubation and reached the highest value at 6 h (Fig. 2). This observation showed that higher temperature is in favor of cathranthine accumulation in roots. The young developmental stage of *C. roseus* roots used in this experiment accounts for the lower content of catharanthine. It is thought that catharanthine content in root is up-regulated with age. The presence of transport of catharanthine from root to leaves might be one reason for the variation in roots during stress.

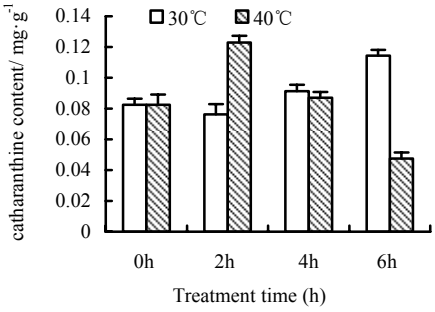

Fig. 2 Catharanthine content in root of *Catharanthus roseus* seedling incubated with different temperature.

Error bars represent ±SD calculated from four independent experiments.

Effect of temperature on alkaloid metabolism in long-term treatment

Vindoline and catharanthine showed similar metabolic properties during treatment. In control leaves, the contents of catharanthine (Fig. 3 b) and vindoline (Fig 3. a) were 1.5 mg·g^{-1} and 2 mg·g^{-1}, respectively, and the variable amount was about 1 mg·g^{-1} during incubation. Concentrations of monomeric alkaloids were higher at 20ºC than at 25ºC and had a sharp increase under the condition of 35ºC. Vindoline and catharanthine attained their peak contents of 8.56 mg·g^{-1} and 6.36 mg·g^{-1}, respectively, at the 15th day.

The result of vinblastine was distinct to its precursors, but the characteristics in different temperature surroundings were similar. Vinblastine content had a peak value for each temperature treatment and appeared at different treatment time. In general, the higher the temperature, the earlier the peak value of vinblastine content appears. The peak value of vinblastine content was about 0.041 mg·g^{-1} at 35ºC and was only about 0.03 mg·g^{-1} at 20ºC or 25ºC (Fig. 3c). It is obvious that accumulation of vinblastine was

not controlled by its precursors precisely. Another remarkable rule of vinblastine changes during the treatment of different temperatures was that vinblastine contents were similar at 15 d exposure irrespective to the preceding discrepancy. The changing way was related with the role of vinblastine during interactions of plants with surrounding temperature.

Vincristine is a derivative of vinblastine and it has very low level in leaves. There is almost no available information on the metabolism and biological function of vincristine in *C. roseus* seedlings. In this study, continuous enhancement of vincristine content characterized the effect of temperature on vincristine. Vincristine content had a decrease trend from 4th day to 13th day under the condition of 35ºC, then had a subsequently rapid increase, attaining 0.027 $mg \cdot g^{-1}$ at 16th day, higher than those in the other conditions (Fig 3 d).

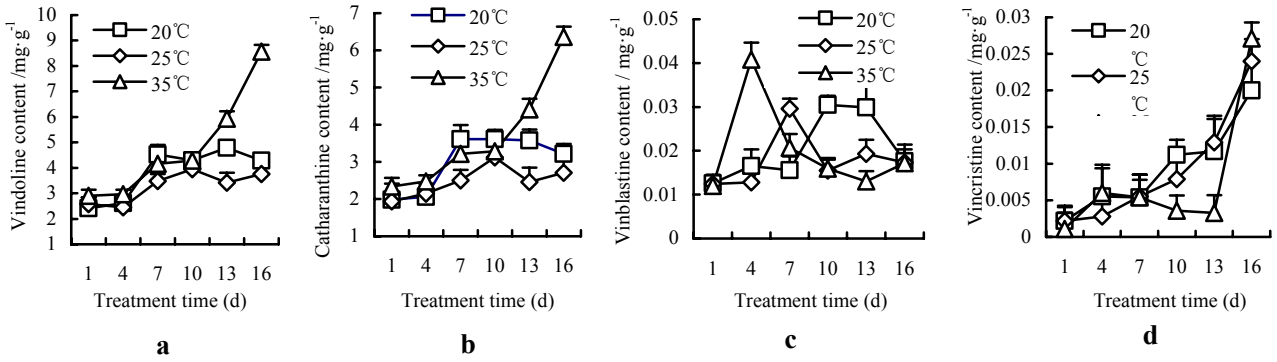

Fig.3 Alkaloid metabolism of *Catharanthus roseus* seedling incubated with different temperatures.

Error bars represent ±SD calculated from four independent experiments.

Discussion and conclusions

There are many kinds of alkaloids in *C. roseus* seedlings and their responding way to stress is not uniform (De Luca and St-Pierre 2000). Different kinds of alkaloids should be characteristic of different changing way, representing special functions (Hashimoto and Yamada 1994). This is the strategies of plants under the condition of limited resources after rigid trade-off (Datta and Sricastava 1997). In this study it was showed that vinblastine concentration rose to the highest values separately at the forth day under 35ºC, at the seventh day under 25ºC and at the tenth day under 20ºC but declined to the same level at last in long-term experiment. In addition, the peak values of vinblastine concentration at 25ºC and 20ºC were lower than that at 35℃. These results of long-term treatment demonstrated that vinblastine contents could be promoted by high temperature in considerable short term and then went back to the beginning amount, and vincristine concentration accumulated gradually. It suggested that the lost vinblastine might be responsible for the rise of vincristine. This is entirely different with the changes of its precursors. The contents of vindoline and catharanthine in long-term treatment were increased to a high level under high temperature condition. Therefore, in practice we can improve the contents of vindoline, catharanthine and vincristine through promoting growth temperature for long-term. In our study besides vindoline and catharanthin, vinblastine also accumulated under high temperature during short-term treatment. Although it is unfeasible to elevate vinblastine by long-term treatment, it is feasible to exert short-term high temperature on *C. roseus* seedlings to raise vinblastine concentration. In contrast to vindoline and catharanthine, vinblastine is one kind of alkaloid with difficulty to be induced by biotic factors, such as ABA and JA which activate rapid biosynthesis and accumulation of many secondary metabolites.

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